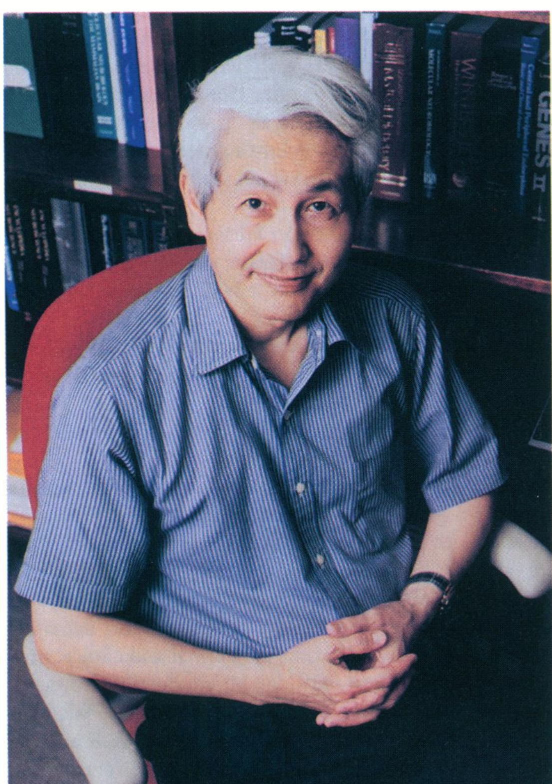
Environmental Toxins and the Brain

For years scientists have suspected that the environment plays a role in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and dementia. Only recently have scientists begun to investigate the complex relationship between environmental toxins and the chronic death of neurons, the cells that serve as information transmitters and processors in the brain.

Two NIEHS researchers, Jean Harry and Jau-Shyong Hong, hope their work will shed light on the mechanisms that trigger neuroimmune responses—eventually killing neurons—in the central nervous system. Ultimately, they hope to uncover drug therapies that could one day slow the progression of neurodegenerative diseases.

Scientists theorize that environmental exposures have a latent effect on the brain, causing a noticeable degeneration only years later. "The nervous system is great in compensation," says Harry, a neurotoxicologist. After a stroke, for example, the victim's surviving neurons sprout and make new connections, striving to keep the brain in balance. As the system ages, however, it gradually forfeits the ability to compensate, and an accelerated aging process may begin.



Steve McCaw, Image Associates, Inc.

Jau-Shyong Hong

"You may have been exposed to something when you were five years old," Harry says. "Basically, the whole process sort of [catches] up with you."

Rogue Brain Cells

Hong and his colleagues in the neuropharmacology section of the Laboratory of Toxicology at the NIEHS have set out to bolster the theory that microglia—brain cells that somehow become activated by neurotoxins or other injuries—kill neurons in the brain. According to Hong, the microglia play a critical surveillance role during the resting stage, just as immune cells do in the peripheral nervous system.

However, when the brain is injured—either through adverse environmental exposure, a viral or bacterial infection, trauma, or stroke—these sentinel cells launch an out-of-control rampage in the brain. Suddenly transformed from loyal bodyguards into overactive destroyers, they kill the very neurons they were intended to protect. Hong likens the process to misuse or overuse of a beneficial drug. Too much of a good thing, he says, can be very harmful. "When [these cells] work too hard, they not only kill the foreign invader, they also kill the neurons," he explains.

Hong's experiments studying reactive gliosis have used an *in vitro* cell culture system. Such a model is necessary, he contends, to reveal molecular pathways involved in this process. Hong's experiments have supported the theory that overactive microglia kill neurons, and that, in contrast, astroglia (large neuroglial cells also known as astrocytes) produce proteins known to promote neuronal growth or survival, which may help to preserve the neurons under attack by inflammatory cells. To further complicate an already complex relationship, microglia are known to release mitogens that activate astroglia, while astroglia produce growth factors that stimulate microgliosis.

Hong's work has also focused on how opioid peptides may modulate the microglia's function. His research team has sought to discover the effects of endogenous opioid peptides on the production of pro-inflammatory cytokines by microglia cells, as well as their possible relationship to toxin-induced neurodegeneration. "We found opioid peptides have a very important effect on microglial function," Hong says. "They potentially could be used as a therapy for the neuroimmune system." Hong explains that opioid peptides tend to dampen the activity of the microglia, helping to prevent the aberrant activation that destroys neurons. According to Hong, information generated from this line of research raises the possibility that inhibition of either deleterious microglial activation or the production of specific microglial-derived neurotoxins could provide therapeutic interventions for neurodegenerative diseases.

Hong's group has also collaborated with a researcher at Duke University Medical Center to examine the role of opioid peptides on the interaction between HIV and microglia. An envelope protein of HIV—gp120—has been shown to activate the microglia, which means that, in an AIDS patient, not only are lymphocytes being destroyed, but brain cells are being destroyed as well. "More than 50% of AIDS patients eventually suffer from AIDS dementia complex," Hong says.

Triggers of Neurodegeneration

Harry, who heads the neurotoxicology group at the NIEHS, suspects that specific patterns of neurodegeneration may be due to neuronal-glia signaling interactions. During the past few years, research on the nervous system has shown that resident cells in the central nervous system can produce cytokines the same way cells in the peripheral nervous system do. These cells can react to injury by initiating a cytokine response, which has been shown to be associated with tissue damage. Injury or inflammation appear to be the precursors of this immune-like response, which could trigger a number of neurodegenerative processes in the brain. Harry's research focuses on understanding the signaling processes associated with this inflammatory response and their role in nervous system damage following exposure to environmental agents.

According to Harry, an important mechanism associated with brain injury is the cytokine inflammatory response. Inflammation at the site of injury, she says, spurs the microglia to release a number of factors, creating a cytokine network that may play a major role in immunologically mediated neurodegenerative diseases. Astroglia produce other growth factors and support the neurons by regulating the neuronal growth and function of microglia.

Harry and her colleagues have completed several experiments aimed at identifying the temporal pattern of pro-inflammatory cytokine responses during chemically induced neurodegeneration. In initial studies, the researchers examined a prototypic model of neurodegeneration through systemic administration of the toxic chemical trimethyltin (TMT) into rats. The TMT caused neuronal necrosis and neurodegeneration in some cells of the hippocampus, while other cells remained unchanged. According to Harry, the question of interest is the pattern of degeneration. "Why do some neurons die while adjacent ones do not?" she asks. "We found that the microglia cells, which have been linked to most of the production of cytokines, co-localized with the neurons that were going to degenerate," Harry explains. "And they co-localized early, prior to seeing any necrosis or any indication of neuronal perturbation."

Harry and her colleagues also observed in those cells an up-regulation—or increased levels—of the cytokines. "So, the microglia and the cytokines, because of their co-localization with neurons, may actually be a driving force in neurodegeneration," Harry proposes.

Harry's team also performed experiments using *in vitro* cultures of glial cells (astrocytes, microglia, and oligodendrocytes) to examine the effects of TMT on morphology and cytokine responses in glia cells alone, without the contribution of neuronal cells. Their data suggest that TMT can directly stimulate glial cells without the neuronal signal. Harry hopes that the *in vitro* system will allow them to look more closely at cellular responses, plus allow researchers to pharmacologically manipulate various glial factors.

The team is currently evaluating a mouse model of TMT that produces a different pattern of hippocampal neuronal damage. The scientists are finding a concentration of microglia around the degenerating neurons, again with a concurrent and most likely related up-regulation of cytokines. These results prompt the question of why the microglia converge on some neurons and leave others within the same region of the brain untouched. Harry hopes to do further research with transgenic animals and genetic mutants to address this question and determine whether the microglia play a neurotoxic role and the astroglia a protective one. She also wants to find out if neuronal degeneration is prevented when pro-inflammatory cytokines, like tumor necrosis factor alpha, are removed from the equation. In short, Harry says, "We're trying to find out if the cytokine response is truly responsible for the neuronal degeneration, or if it augments it, or if it is only an injury response occurring within a similar time period."

The Next Step

Before embarking on the next level of *in vivo* research, the team plans to study an *in vitro* culture system using the same chemical and identical cells from the hippocampus. "That will help us identify which intervention approaches may be successful. Then we can go back *in vivo* to confirm whether we can modulate this response," says Harry.

The *in vitro* approach does have some disadvantages if used without caution, according to Harry. Because the central nervous system is protected by a blood-brain barrier—the body's way of keeping unwanted circulating factors at bay—the brain may be buffered from direct exposure to certain chemicals. "There are ways the brain gets things out, that in culture, it can never get away from," Harry explains. "And the other thing is, you never know exactly what the brain sees," she adds. "If you take a drug or you're exposed to a chemical, it can be biometabolized to another form. [However], the most important limitation is the inability to mimic the complex nature of the nervous system in an isolated *in vitro* system."

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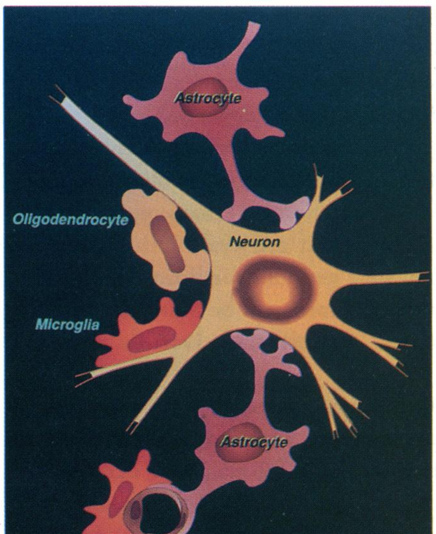
Jean Harry

For this reason, Harry doesn't advocate using cultures as an effective screen for neurotoxicity, arguing that there is no sure way to have all the systems in place to accurately determine a chemical's toxicity. "We don't know the mechanisms of toxicity to make sure we cover all the possibilities," she concedes. "However, [cultures] do offer a system to examine specific mechanistic questions."



Jean Harry

Mind-boggling complexity. By studying the relationships among different brain cells, scientists hope to shed light on the mechanisms of neurodegenerative diseases.

Like Hong, Harry also hopes for a drug to help stem the tide of neurodegeneration within the brain. "It's probably one that's already out there; nothing necessarily new, but one that would work on modulating the immune response," she says. "But we'd first have to confirm that the drug doesn't cause problems in the brain." Here again, she explains, culture systems could pose a problem: "We're finding things that are therapeutic agents that, when given to the person, can have activity in the culture that you don't necessarily know how to interpret."

Researchers continue to understand more about neurodegenerative diseases, and to work steadily toward therapies that could one day preserve neurons in the brains of people who have begun to experience symptoms of neurodegeneration. Preservation is the key, because the cells in the central nervous system can't regenerate like their counterparts in the peripheral nervous system. The brain comes outfitted with a finite number of neurons—billions of them. Harry says, "you've got one shot at getting all of them you need. If anything messes that up, you can work on a lower balance and you may be fine—until you age."

Jennifer Medlin